Since $N\bar{O}$ and $O_2$ are isosteres it is possible that the ground state of the $N\bar{O}$-ion is a triplet state. This state gives a magnetic moment $\mu = 2.83\ \mu_B$. In order to settle this question the metal nitrosyls LiNO, NaNO and KNO were prepared and measured magnetically; LiNO was prepared for the first time. The results of the chemical analyses and the magnetic measurements are given in Table 1. The results prove that $N\bar{O}$ is in a singlet state — if present in these compounds.

*Table 1.* Analyses and diamagnetic susceptibilities of alkali metal nitrosyls.

| Substance | % Metal calc. | % Metal found | $\chi_g \times 10^6$ | $\chi_M \times 10^6$ | $\chi_{N\bar{O}} \times 10^6$ |
|---|---|---|---|---|---|
| LiNO | 18.78 | 18.50 | $-0.56_2$ | −20.8 | −20.1 |
| NaNO | 43.37 | 42.98 | $-0.40_0$ | −21.2 | −17.2 |
| KNO | 56.57 | 56.42 | $-0.46_4$ | −32.1 | −18.1 |

The metals were determined as sulphates. The metal nitrosyls were measured magnetically in evacuated glass tubes (Gouy method) at 20—22°C.

The gram ionic susceptibilities for $NO^-$ were calculated from the $\chi_M$ values by means of the gram ionic susceptibilities $-0.7 \times 10^{-6}$, $-4 \times 10^{-6}$ and $-14 \times 10^{-6}$ for the ions $Li^+$, $Na^+$ and $K^+$, respectively. *A reasonable mean value for the gram ionic susceptibility of* $NO^-$ *is* $-18 \times 10^{-6}$. This number is calculated from the 3 values in Table 1 giving the values for NaNO and KNO double weight because the analytical data for these substances are in better accordance with the theoretical values than is the case for LiNO.

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# On the Existence of Oscillating Enzymatic Reactions and on a Possible Interpretation of Spontaneous Spike Potentials in Nerves

J. A. CHRISTIANSEN

*Københavns Universitets Fysisk-Kemiske Institut, Copenhagen, Denmark*

An overall reaction

$$A_1 + A_2 + A_3 \rightleftharpoons B_2 + B_3 + B_1 \qquad (B)$$

is supposed to be catalyzed by an enzyme according to the mechanism (A)

$$\begin{aligned} A_1 + X_1 &\rightleftharpoons X_2 + B_2 \quad (\pm 1) \\ A_2 + X_2 &\rightleftharpoons X_3 + B_3 \quad (\pm 2) \\ A_3 + X_3 &\rightleftharpoons X_1 + B_1 \quad (\pm 3) \end{aligned} \qquad (A)$$

where $X_1$, $X_2$, $X_3$ are three different forms of the enzyme or complexes thereof with the substrates ($A_i$, $B_i$). The sum of the concentrations of the three forms is the total constant enzyme concentration $E$, which is supposed to be stoichiometrically small as compared to the concentrations of the substrates. Let $x_1$, $x_2$ and $x_3$ be the fractions of the enzyme present in the three states, and let $s_1$, $s_2$ and $s_3$ be the resulting rates of the three partial reactions, divided by $E$.

We then have the four equations

$$\begin{aligned} x_1 w_1 - x_2 w_{-2} &= s_1 \\ x_2 w_2 - x_3 w_{-3} &= s_1 \\ x_3 w_3 - x_1 w_{-1} &= s_2 \end{aligned} \qquad (1)$$

$$x_1 + x_2 + x_3 = 1 \qquad (2)$$

where $w_1$ is the probability in unit time for $X_1$ to react according to $(+1)$ in (A), $w_{-1}$ the probability for $X_2$ to react with $B_2$ according to $(-1)$ in (A), *etc.*

The steady state solution of the system (1) in connection with the condition (2) is well known [12]. It is characteristic for this, that if the probabilities $w$ are exactly known the result of the calculation is of unlimited accuracy.

There exists, however, another solution of (1) and (2) for which this is not true in so far as in this case the (weighted) sums of the squared deviations from mean values have well defined constant values, different from zero.

This solution seems therefore to be inherently more probable than the usual one.

The essential points in the derivation are: 1) the introduction of a system of "weights" $m_1$, $m_2$ and $m_3$; 2) the description of the deviations as vectors.

The weights are defined in terms of the $w$'s by the "auxiliary equations" (3).

$$\begin{aligned} m_1w_1 - m_3w_{-3} &= 1 \\ m_2w_2 - m_1w_{-1} &= 1 \\ m_3w_3 - m_2w_{-2} &= 1 \end{aligned} \tag{3}$$

whose solutions may be written in the form (4)

$$\begin{aligned} (1-\beta)m_1w_1 &= 1 + \beta_2\beta_3 + \beta_3 \\ (1-\beta)m_2w_2 &= \beta_1 + 1 + \beta_3\beta_1 \\ (1-\beta)m_3w_3 &= \beta_1\beta_2 + \beta_2 + 1 \end{aligned} \tag{4}$$

where $\beta_i = w_{-i}/w_i$ and $\beta = \beta_1\beta_2\beta_3$

If $\beta$ is small as compared to 1 the overall reaction (B) is irreversible, which we shall assume.

We define further a set $g_1$, $g_2$ and $g_3$ of pure numbers by

$$g_1 = m_iw_i - 1/2 \tag{5}$$

The calculations which will be published later, then lead to the following description of the deviations of $x_1$, $x_2$ and $x_3$ from their mean values $\lambda_1$, $\lambda_2$ and $\lambda_3$.

1) Draw a triangle with the sides $\lambda_1$, $\lambda_2$ and $\lambda_3$ where

$$\begin{aligned} \lambda_1 &= A\sqrt{g_2 + g_3} \\ \lambda_2 &= A\sqrt{g_3 + g_1} \\ \lambda_3 &= A\sqrt{g_1 + g_2} \end{aligned} \tag{6}$$

$A$ is determined by the condition that the perimeter of the triangle $\lambda_1 + \lambda_2 + \lambda_3$ shall equal 1.

2) Project the sides of the triangle on a pointer which rotates in the direction 123 with the constant angular velocity $2\pi\nu$.

These projections then represent the (real) deviations from the fixed values $\lambda_1$, $\lambda_2$ and $\lambda_3$, respectively. The (real) sum of the deviations is evidently zero, but separately at most one of them can be zero at a certain time.

To calculate $\nu$ we use the relations

$$\begin{aligned} dq_1/dt &= \sigma_3 - \sigma_1 \\ dq_2/dt &= \sigma_1 - \sigma_2 \\ dq_3/dt &= \sigma_2 - \sigma_3 \end{aligned} \tag{7}$$

and their complex conjugates. $q_1$ is the (complex) deviation of $x_i$ from $\lambda_i$ and $\sigma_i$ the (complex) deviation of $s_i$ from $s$. Eqns. (7) follow directly from the law of conservation of matter.

(By use of (3) and the condition (2) it can be shown that

$$m_1s_1 + m_2s_2 + m_3s_3 = s(m_1 + m_2 + m_3) = 1 \tag{8}$$

The first equation (8) shows that $s$ is the weighted mean of the different possible values of $s_i$ and the second that this mean is exactly the steady state value. It should be noted that our mean values $\lambda$ are different from the usual steady state values, the reason being that the system is never in its steady state.

The real frequency $\nu$ can then be calculated from

$$8\pi g\nu = (g_1+g_2)/m_1 + (g_2+g_3)/m_3 + (g_3+g_1)/m_3 \tag{9}$$

where

$$g^2 = g_1g_2 + g_2g_3 + g_3g_1 \tag{10}$$

By use of (5) eqn. (9) may be simplified into

$$8\pi g\nu = (w_1+w_{-1}+w_2+w_{-2}+w_3+w_{-3}) \tag{11}$$

Finally to arrive at the actual values of $x_1$, $x_2$ and $x_3$ we simply add to the sides of the fixed triangle $\lambda_1$, $\lambda_2$, $\lambda_3$ the respective varying deviations from our geometrical construction. The sum of the resulting lengths will then be constant, but they will vary periodically with time. The lengths separately will represent the distribution on the different forms of the enzymes at different times. Considering $x_1$ it will be seen that it performs harmonic oscillations between zero and $2\lambda_1$, and similarly for the others, but the oscillations are never in the same phase.

Now these oscillations may be registered by an oscillograph as spike potentials [3-5] if the enzymatic system is electrochemically active.

The source of the periodically occurring potentials is usually believed to be located in some "end-organ" from which they are transmitted through the nerve.

Such spike-potentials may perhaps not be expected to be generally observable in vessels of macroscopic dimension as the whole spectrum then may be diffuse. This is because of the vague definition of time "zero" in such cases. But in nerves whose diameters are of the order of magnitude 1 $\mu$ no such difficulty arises. It may therefore be, that we should assume the presence of an enzyme which catalyses some irreversible process, perhaps in the membrane of the end-organ or / and of the nerve.

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# On Hydroxylamine Compounds in *Azotobacter* Cultures. I. Formation of Hydroxylamine Compounds

NILS-ERIK SARIS and ARTTURI I. VIRTANEN

*Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland*

Blom [1] was the first to demonstrate small amounts of hydroxylamine in nitrogen-fixing *Azotobacter* cultures, and later on bound hydroxylamine was found by Endres [2]. Hydroxylamine was formed only from nitrate or molecular nitrogen but not from ammonium nitrogen. On the other hand Virtanen and Hakala [3] observed that about the same amount of bound hydroxylamine was found in the nutrient solutions of *Azotobacter vinelandii* both when nitrate nitrogen, ammonium nitrogen, and molecular nitrogen were the sources of nitrogen. Virtanen and Järvinen [4] investigated the formation of bound hydroxylamine in the cell suspension of *Azotobacter vinelandii* and found that it was formed both from $N_2$-, $NO_3$-, and $NH_4$-N, although more rapidly from molecular nitrogen and nitrate nitrogen than from ammonium nitrogen. These authors determined bound hydroxylamine in the cell mass separated by centrifugation.

The present authors have now further studied the formation of hydroxylamine compounds found in the nutrient solution of *Azotobacter*. Under the experimental conditions used the enrichment of hydroxylamine compounds in cells was too scant to make a closer investigation of these compounds possible. On the other hand bound hydroxylamine was enriched in the nutrient solution in such amounts that a closer investigation of them could be attempted.

*Nutrient solution.* 1.5 g Burk's salt, 10 mg $Fe_2(SO_4)_3$, 15 mg $FeSO_4$, $7H_2O$, 0.25 mg $Na_2MoO_4$ per litre, pH 7.2. In the experiments proper glycerol was the source of carbon (*cf.* below); the source of nitrogen is mentioned in connection with the experimental results.

*Determination of bound hydroxylamine.* Csáky's method was used, with the modification that the hydrolysis time was shortened to one hour. To destroy nitrite a pinch of urea was added. Determinations were made both from 2 and 10 ml.

*Azotobacter strain.* In preliminary experiments the largest amount of bound hydroxylamine was found in cultures of *Azotobacter chroococcum* K 1. In cultures of other investigated strains (*A. vinelandii* Kluyver, *vinogradsky* Kluyver, *beijerinckii*, and a not closer characterized strain isolated from soil) a much smaller amount of bound hydroxylamine, if any, was enriched.

*Effect of the carbon source.* Because Endres obtained the largest amount of bound hydroxylamine when lactate was the source of carbon, growth experiments were performed with different carbon compounds (glycerol, Ca-lactate, saccharose, ethanol, acetone, sorbitol). In the concentrations investigated glycerol was as good a carbon source as lactate, and it was used in the experiments proper. The large amounts of hydroxylamine found by Endres in lactate nutrient solutions were not, however, even approximately obtained. He found maximum $2 \times 10^{-4}$ mole $NH_2OH$ per 10 ml, the present authors about $10^{-5}$ only. This circumstance made the isolation of hydroxylamine compounds and their characterization difficult.

*Cultivation methods. Method 1. Azotobacter* was allowed to grow without shaking in 1 litre flat flasks containing 200 ml of nutrient solution each. 200 ml of nutrient solution without combined nitrogen was inoculated with 2.5 ml of a 4-day-old *Azotobacter* culture. The experiment was terminated after 3 days, the maximum amount of bound hydroxylamine being then found in the solution. The influence of the age of the cultures on the content of bound hydroxylamine in the nutrient solution can be seen from the following results with *Azotobacter chroococcum* K 1: